

Case No.: ITT0039YP  
U.S.N.: 10/500,971  
Filed: 2/16/05

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 45/00, 45/05, 47/00</b>		<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 00/13708</b>
			<b>(43) International Publication Date:</b> 16 March 2000 (16.03.00)
<b>(21) International Application Number:</b> PCT/US99/20402		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
<b>(22) International Filing Date:</b> 3 September 1999 (03.09.99)			
<b>(30) Priority Data:</b> 60/099,146      4 September 1998 (04.09.98)      US			
<b>(71) Applicant (for all designated States except US):</b> VI-ROPHARMA INCORPORATED [US/US]; 405 Eagleview Boulevard, Exton, PA 19341 (US).			
<b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> BAILEY, Thomas, R. [US/US]; 217 Amanda Lane, Phoenixville, PA 19460 (US). YOUNG, Dorothy, C. [US/US]; 5 Ironwood Drive, Collegeville, PA 19426 (US).		<b>Published</b> <i>With international search report.</i>	
<b>(74) Agents:</b> HAGAN, Patrick, J. et al.; Dann, Dorfman, Herrell and Skillman, Suite 720, 1601 Market Street, Philadelphia, PA 19103-2307 (US).			
<b>(54) Title:</b> METHODS FOR TREATING OR PREVENTING VIRAL INFECTIONS AND ASSOCIATED DISEASES			
<b>(57) Abstract</b>  Methods are provided for the treatment and prophylaxis of viral infection and disease associated with such infection.			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

**METHODS FOR TREATING OR PREVENTING  
VIRAL INFECTIONS AND ASSOCIATED DISEASES**

Thomas R. Bailey  
Dorothy C. Young

**FIELD OF THE INVENTION**

The present invention relates to methods for preventing or treating viral infections and the diseases associated therewith, particularly those viral infections and associated diseases caused by  
5 viruses within the Flaviviridae family.

**BACKGROUND OF THE INVENTION**

The Flaviviridae family consists of three genera and several viruses that are currently unassigned to  
10 specific genera. The hepacivirus genus includes the hepatitis C viruses (HCV). Viruses such as GB virus-A and GB virus-A-like agents, GB virus-B and GBV-C or hepatitis G virus, while at present not formally  
15 classified within the hepacivirus genus, are closely related to HCV and represent unassigned members of the Flaviviridae family. Also within the Flaviviridae is the pestivirus genus, which includes bovine viral  
diarrhea viruses (BVDV), border disease viruses and classical swine fever virus, and the flavivirus genus,  
20 with viruses such as dengue, yellow fever, Japanese encephalitis and tick-borne encephalitis viruses.

Viruses within this family cause significant disease in human and animal populations. HCV is a major cause of human hepatitis globally. The World  
25 Health Organization estimates that 170 million people worldwide are presently infected with the virus. Most infections become persistent and about 60% of cases develop chronic liver disease. Chronic HCV infection can lead to development of cirrhosis, hepatocellular  
30 carcinoma and liver failure.

Interferon and interferon in combination with ribavirin are used in the U.S. for hepatitis due to HCV. These treatments are associated with improved serum enzyme response in some patients. The remainder  
5 are non-responsive to treatment. For responders, a sustained clinical improvement is seen in only a small percentage of patients; the majority of patients relapse upon cessation of treatment. Thus, the effectiveness of therapy for chronic hepatitis C is  
10 variable and its cure rate remains low. Moreover, therapy is often associated with considerable side effects.

Pestivirus infections of domesticated livestock cause significant economic losses worldwide.  
15 Pestiviruses cause a range of clinical manifestations including abortion, teratogenesis, respiratory problems, chronic wasting disease, immune system dysfunction and predisposition to secondary viral and bacterial infections. Certain BVDV strains cause an  
20 acute fatal disease. BVDV can also establish persistent infections in fetuses. When born, these persistently infected (PI) animals remain viremic throughout life and serve as continuous virus reservoirs. PI animals often succumb to fatal mucosal  
25 disease.

Flaviviruses are important pathogens of man and are also prevalent throughout the world. There are at least 38 flaviviruses associated with human disease, including the dengue fever viruses, yellow fever virus  
30 and Japanese encephalitis virus. Flaviviruses cause a range of acute febrile illnesses and encephalitic and hemorrhagic diseases.

Currently, there are no antiviral pharmaceuticals

to prevent or treat pestivirus or flavivirus infections.

New therapies and preventatives are clearly needed for infections and diseases caused by viruses of Flaviviridae family.

In considering approaches to the diagnosis, control, prevention and treatment of infections and associated diseases caused by viruses, it is often desirable to identify virus-specific functions that may be exploited in such approaches. In particular, enzymatic activities of virus-encoded polypeptides are quite useful. These virus-specified components are often essential for virus replication and may be suitable targets for antiviral drug discovery strategies.

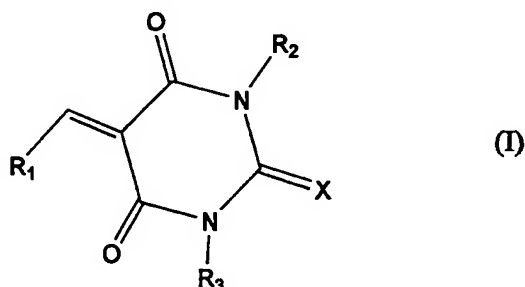
One such target that plays a central role in the life cycle of many RNA viruses is the virus-encoded RNA-dependent RNA polymerase (RdRp) protein. Regarding viruses of the Flaviviridae, this protein is termed NS5B in the case of the hepaciviruses and pestiviruses, and NS5 in the case of the flaviviruses (collectively referred to as NS5). RdRp proteins are a key component of the virus replicase complex, enabling the virus to replicate its RNA genome and produce progeny viruses. The RdRp of RNA viruses is an attractive target for antiviral drug development.

### 30 SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a method for treating viral infection and diseases associated with such infection in a living

host having such infection, by administering to such host a therapeutically effective amount of a compound, or a precursor thereof, having the following formula:

5



wherein X represents a moiety selected from the group consisting of S, O, or NR<sub>a</sub>, R<sub>a</sub> being hydrogen or alkyl of 1-5 carbon atoms;

- 10        R<sub>1</sub> represents a radical selected from those consisting of an unsubstituted or substituted heterocyclic group, an unsubstituted or substituted bicyclic ring moiety, an unsubstituted or substituted phenyl (C<sub>6</sub>H<sub>5</sub>) group, an unsubstituted or substituted
- 15        biphenyl (C<sub>6</sub>H<sub>5</sub>-C<sub>6</sub>H<sub>4</sub>) group, an unsubstituted or substituted ω-phenylalkenyl (C<sub>6</sub>H<sub>5</sub>(CH=CH)<sub>n</sub>) group, n being an integer from 1-5, such as 2-phenylethenyl, an unsubstituted or substituted ω-phenylalkynyl (C<sub>6</sub>H<sub>5</sub>(C≡C)<sub>p</sub>) group, p being an integer from 1 to 5, or an
- 20        unsubstituted or substituted alkyl group of 1-5 carbon atoms which may be straight or branched chain, said heterocyclic group being selected from those consisting of furan, thiophene, oxazole, oxadiazole, pyridine, pyrimidine, pyrazole, triazole, pyridazine,
- 25        1,3-oxathiolane, thiazole, thiadiazole, imidazole,

pyrrole, tetrazole and triazine, said bicyclic ring moiety being selected from those consisting of benzofuran, isobenzofuran, benzothiophene, isobenzothiophene, benzoxazole, benzopyrrole, 5 isoindole, benzpyrazole, quinoline, isoquinoline, 1,2-benzodiazine, 1,3-benzodiazine, 1,2,3-benzotriazole, benzothiazole, benzimidazole, 1,2,3-benzotriazine and 1,2,4-benzotriazine, the heterocyclic group and bicyclic ring moiety substituents being selected from 10 those consisting of alkyl of 1-5 carbon atoms, halogen, alkoxy, hydroxy, nitro, or an unsubstituted or substituted phenyl group;

$R_2$  and  $R_3$  independently represent hydrogen, an unsubstituted or substituted phenyl group, or 15  $-(CH_2)_qCOOH$ ,  $q$  being an integer from 1-5, and at least one of said  $R_2$  and  $R_3$  being hydrogen;

the phenyl group substituents, the biphenyl group substituents, the  $\omega$ -phenylalkenyl group substituents and the  $\omega$ -phenylalkynyl group substituents being at 20 least one selected from those consisting of halogen, nitro, carboxy, hydroxy, alkyl of 1-5 carbon atoms, trifluoromethyl, alkoxy, acyloxy, cyano, amino, alkylamino, dialkylamino, sulfonamido, carboxamido, carbalkoxy, thio, alkylthio, alkylsulfinyl and 25 alkylsulfonyl; the alkyl group substituents being at least one selected from those consisting of carboxy, hydroxy, alkoxy, amino, alkylamino, dialkylamino, thio or alkylthio, and the isomers and pharmaceutically acceptable salts of said compound.

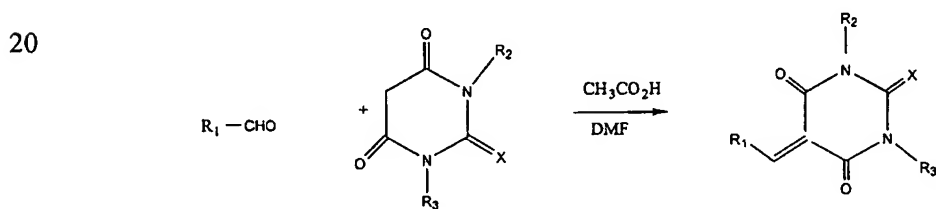
30 In the compounds of formula I, above, the substituents of the heterocyclic group, the bicyclic

ring moiety, the phenyl group, the biphenyl group, etc. may also be perhaloalkyl, dihaloalkyl, monohaloalkyl, and the heterocyclic group and bicyclic ring moieties may also be substituted with thio, alkylthio, alkylsulfinyl or alkylsulfonyl substituents.

The above compounds may also be used for prevention of viral infections and disease associated with such infection in a susceptible host by administering to the host a prophylactically effective amount of the above-described compound or precursor thereof.

#### DETAILED DESCRIPTION OF THE INVENTION

The compounds used in the method of the invention can be conveniently prepared from known starting materials according to the general synthetic scheme illustrated below, among other methods.



25 Preparations of specific anti-viral compounds which may be used in the practice of this invention are exemplified below.

In carrying out the above general synthetic scheme, the appropriate aldehyde is reacted with barbituric acid or 2-thiobarbituric acid in a mixture of dimethyl formamide (DMF) and acetic acid at  $80^\circ C$  or in DMF and hydrochloric acid at room temperature. Alternatively, the reaction may be carried out using



5 piperidine in ethanol as the reaction medium. The aldehyde starting materials, or acids from which they may be converted, e.g., by chemical reduction, are available from various commercial sources (e.g., Sigma, St. Louis, MO).

Other compounds useful in the practice of this invention may be similarly prepared, substituting the appropriate starting material(s) in the above reaction scheme.

10 *In vitro* studies have demonstrated the usefulness of compounds described herein as antiviral agents. Antiviral activity was measured by the inhibitory activity of the compounds against viral RdRp in an enzymological assay for RNA synthesis.

15 All possible isomers of formula I, above, are within the scope of the present invention. Representative examples of such isomers include, without limitation, cis and trans isomers.

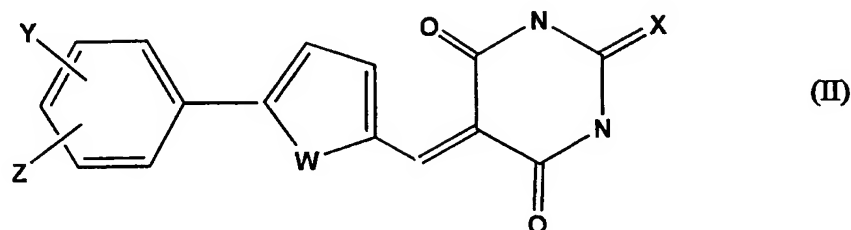
20 The term "alkyl" as used herein refers to aliphatic hydrocarbon radicals of one to five carbon atoms in length. Similarly, the term "alkyl", or any variation thereof, used in combination form to name substituents such as alkoxy, alkylamino, or the like also refers to aliphatic hydrocarbon radicals of one  
25 to five carbon atoms in length.

The term "carboxamido", as used herein, refers to a radical or substituent of the formula  $-C(=O)-NR''R'''$ , wherein  $R''$  and  $R'''$  represent hydrogen or alkyl.

30 The term "sulfonamido", as used herein, refers to a radical or substituent of the formula  $-SO_2NR''R'''$  or  $-NR''-SO_2R'''$ , wherein  $R''$  and  $R'''$  are as previously defined.

Particularly useful in the practice of this

invention are compounds, including isomeric forms, having the formula:



5 in which Y and Z may be hydrogen, halogen, nitro, carboxy, hydroxy, alkoxy, alkyl of 1-5 carbon atoms, trifluoromethyl, trifluoromethoxy, acyloxy, cyano, sulfonamido, carboxamido, carbalkoxy, thio, alkylthio, alkylsulfinyl, alkylsulfonyl, amino, alkylamino or  
10 dialkylamino and W may be -O-, -S- or -N(Rb)-, Rb being hydrogen or alkyl of 1-5 carbon atoms, X is as previously defined, and the isomers and pharmaceutically acceptable salts of said compounds.

The compounds of formulas I and II above and  
15 their pharmaceutically acceptable salts exhibit antiviral activity. The method of the invention is particularly effective against viruses of the Flaviviridae family and is useful in treating and/or preventing infections and diseases associated with  
20 these viruses in living hosts.

The compounds described above or precursors (e.g., prodrugs) thereof and their pharmaceutically acceptable salts are also useful in treating and preventing viral infections and diseases in living  
25 hosts when used in combination with supplemental active agents, including but not limited to interferons, ribavirin, protease inhibitors,

immunoglobulins, immunomodulators, hepatoprotectants, anti-inflammatory agents, antibiotics, antivirals, anti-infectious agents, and the like.

Compounds described herein are also useful in preventing or resolving viral infections in cell, tissue or organ cultures and other *in vitro* applications. For example, inclusion of compounds of the invention as a supplement in cell or tissue culture growth media and cell or tissue culture components will prevent viral infections or contaminations of cultures not previously infected with viruses. Compounds described above may also be used to eliminate viruses from cultures or other biological materials infected or contaminated with viruses (e.g., blood), after a suitable treatment period, under any number of treatment conditions as determined by the skilled artisan.

Some of the compounds used in the method of the invention such as those containing basic substituents can form useful salts with various inorganic and organic acids, including, without limitation, hydrochloric or acetic acid, and those compounds containing acidic functionalities can form salts with inorganic and organic bases, including, without limitation, alkali metal hydroxides, alkaline earth metal hydroxides, piperidine, ammonium hydroxide, triethylamine or the like.

The pharmaceutically acceptable salts of the compounds of formulas I and II are prepared following procedures that are familiar to those skilled in the art.

The antiviral pharmaceutical compositions used in the method of the present invention comprise one or

more of the compounds of formula I or II, above, as the active ingredient, and, optionally, at least one supplemental active agent, in combination with a pharmaceutically acceptable carrier medium or auxiliary agent.

The composition may be prepared in various forms for administration, including tablets, caplets, pills or dragees, or can be filled in suitable containers, such as capsules, or, in the case of suspensions, filled into bottles. As used herein, "pharmaceutically acceptable carrier medium" includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's Pharmaceutical Sciences, Fifteenth Edition, E.W. Martin (Mack Publishing Co., Easton, PA, 1975) discloses various carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is incompatible with the antiviral compounds used in practicing the invention; such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

In the pharmaceutical compositions of the invention, the active agent may be present in an amount of at least 0.5% and generally not more than 90% by weight, based on the total weight of the composition, including carrier medium and/or

supplemental active agent(s), if any. Preferably, the proportion of active agent varies between 2-50% by weight of the composition.

Pharmaceutical organic or inorganic solid or liquid carrier media suitable for enteral or parenteral administration can be used to make up the composition. Gelatine, lactose, starch, magnesium stearate, talc, vegetable and animal fats and oils, gum, polyalkylene glycol, or other known carriers or excipients for medicaments may all be suitable as carrier media or excipients.

The compounds described above may be administered using any amount and any route of administration effective for attenuating infectivity of the virus. Thus, the expression "amount effective to attenuate virus infectivity", as used herein, refers to a nontoxic but sufficient amount of the antiviral agent to provide the desired treatment of viral infection. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the individual patient, the severity of the infection, the particular antiviral agent and its mode of administration, and the like.

The antiviral compounds are preferably formulated in dosage unit form for ease of administration and uniformity of dosage. "Dosage unit form" as used herein refers to a physically discrete unit of antiviral agent appropriate for the patient to be treated. Each dosage should contain the quantity of active material calculated to produce the desired therapeutic effect either as such, or in association with the selected pharmaceutical carrier medium. Typically, the antiviral compounds will be

administered in dosage units containing from about 0.1 mg to about 500 mg of the antiviral agent by weight of the composition, with a range of about 1 mg to about 100 mg being preferred.

5           The antiviral compounds described herein may be administered as such, or in the form of a precursor from which the active agent can be derived, such as a prodrug. A prodrug is a derivative of a compound described herein, the pharmacologic action of which  
10 results from the conversion by chemical or metabolic processes *in vivo* to the active compound. Prodrugs include, without limitation, esters of the compounds of Formulas I or II, above, having carboxyl or hydroxyl functionalities. Such esters may be prepared  
15 from simple or functionalized aliphatic alcohols or carboxylic acids. Such prodrugs may be prepared according to procedures well known in the field of medicinal chemistry and pharmaceutical formulation science.

20           The antiviral compounds may be administered orally, rectally, parenterally, such as by intramuscular injection, subcutaneous injection, intravenous infusion or the like, intracisternally, intravaginally, intraperitoneally, locally, such as by  
25 powders, ointments, drops or the like, or by inhalation, such as by aerosol or the like, depending on the nature and severity of the infection being treated. Depending on the route of administration, the compounds of the invention may be administered at  
30 dosage levels of about 0.001 to about 120 mg/kg of subject body weight per day and preferably from about 0.01 to about 30 mg/kg of subject body weight per day, one or more times a day, to obtain the desired

therapeutic effect.

By way of example, a suitable dose for oral administration would be on the order of 30 mg/kg of body weight per day, whereas a typical dose for  
5 intravenous administration would be on the order of 10 mg/kg of body weight per day.

These antiviral compounds will typically be administered from 1 to 4 times a day so as to deliver the above-mentioned daily dosage. However, the exact  
10 regimen for administration of the compounds and compositions described herein will necessarily be dependent on the needs of the individual host being treated, the type of treatment administered and the judgment of the attending medical specialist. As used  
15 herein, the terms "host" includes both humans and animals.

In view of the inhibitory effect on viral RNA synthesis produced by the compounds used in the method of the invention, it is anticipated that these  
20 compounds will be useful not only for therapeutic treatment of virus infection, but for virus infection prophylaxis, as well. The dosages may be essentially the same, whether for treatment or prophylaxis of virus infection.

25 The following examples are provided to describe the invention in further detail. These examples, which set forth a preferred mode presently contemplated for carrying out the invention, are intended to illustrate and not to limit the invention.

30 The examples below illustrate the chemical synthesis of compounds used in the method of the invention.

**EXAMPLE 1**

5- (2,4-Dihydroxyphenylmethylene) -2-

**thioxodihydropyrimidine-4,6-dione** - To a solution of 0.144 g (1 mmol) of 4,6-dihydroxy-2-mercaptopyrimidine and 2 ml of acetic acid in 2 ml of DMF was added a solution of 2,4-dihydroxybenzaldehyde (1 mmol) in 2 ml of DMF. The suspension was heated to 80°C under nitrogen for 16 hours. Upon cooling to room temperature, the reaction was poured into water and the precipitate collected by filtration. The crude product was washed with water, and the solid boiled in methanol for 1 minute. Filtration provided 2.6 mg of product.

**EXAMPLE 2**

5- (5- (2-Trifluoromethylphenyl) furan-2-ylmethylene) -2-

**thioxodihydropyrimidine-4,6-dione** - A suspension of 0.242 g (1 mmol) of 5- (2-trifluoromethylphenyl) furfural, 0.144 g (1 mmol) of 4,6-dihydroxy-2-mercaptopyrimidine, and a drop of piperidine in 6 ml of ethanol was stirred with heating at 80°C for 4 hours. Upon cooling, the orange suspension was poured into 50 ml of water and sonicated for 10 minutes. Filtration of the orange solvent and drying provided 0.204 g of the product as an orange powder.

**EXAMPLE 3**

5- (5-bromothiophen-2-ylmethylene) -2-

**thioxodihydropyrimidine-4,6-dione** - In a procedure identical to Example 1, 0.144 g (1 mmol) of 4,6-dihydroxy-2-mercaptopyrimidine, and 0.207 g (1 mmol) of 5-bromothiophenecarboxaldehyde in a solution of 2



ml of glacial acetic acid and 4 ml of DMF was heated at 80°C for 12 hours. Upon cooling to room temperature, the reaction was poured into water and the precipitate collected by filtration. The crude product was washed with water, and the solid boiled in methanol for 1 minute. Filtration provided 92 mg of product as an orange solid.

#### EXAMPLE 4

10 5-(5-(5-[3,4-dichlorophenyl]furan-2-yl-methylene)pyrimidine-2,4,6-trione

a) To a solution of 6.03 gm (0.0273 moles) of 5-bromo-2-furancarboxaldehyde dimethylacetal in 75 mg of dry THF at -78°C under argon was added 12 ml (1.1 eq) of 25 M n-butyl lithium. After 10 minutes, the yellow solution was quenched with 8.88 g (1 eq) of tributyltin chloride, and the reaction slowly allowed to warm to room temperature. After extraction of the solution with water, drying the organic layer over anhydrous sodium sulfate and removal of the solvent, 11.3 g of 5-tributyltin-2-furancarboxaldehyde dimethylacetal was obtained as a reddish oil.

b) To a solution of 2.38 g (10.5 mmoles) of 1-bromo-3,4-dichlorobenzene and 5.0 g (11.6 mmoles) of the product of step a), above, in 25 ml of dry tetrahydrofuran was added 356 mg palladium (II) chloride di(triphenylphosphine) and the solution was heated to reflux under argon. The solution slowly turned dark brown and was left at reflux temperature for 12 hours. After cooling to room temperature, the solution was diluted with ether and extracted twice with water. The aqueous layer was extracted with ether and the combined organic layer was dried and

decolorized with charcoal. The solvent was removed and the residue was dissolved in ethyl acetate and the solution passed through a silica gel column. The solution was concentrated to dryness and the residue dissolved in a solution of 95% hexane and 5% ethyl acetate and passed through a silica gel HPLC column. Fractions were collected and those fractions, which were pure by tlc, were combined and recrystallized from a mixture of ethyl acetate/hexane to give 245 mg of 5-(3,4-dichlorophenyl) furan-2-yl-carboxaldehyde.

c) To a mixture of 100 mg (.415 mmoles) of the furan carboxaldehyde prepared in step b), above, and 56 mg (.436 mmoles) of barbituric acid in 10 ml of ethanol was added 2 drops of piperidine and the solution was heated to reflux. An orange solid began to form. The mixture was kept at reflux for 5 minutes and then allowed to cool to room temperature. The mixture was poured into water and the solid collected, washed with water and hexane and dried. After drying, 330 mg of the title compound was obtained.

#### EXAMPLE 5

##### Inhibition of Viral RNA Replication

The discovery of inhibitors of viral polymerases and related proteins generally requires the evaluation of large numbers of chemical compounds or mixtures of chemical compounds. Thus, an assay for the polymerase activity that is capable of high volume screening, in other words, a high-throughput assay, is desirable. There are a variety of assay methodologies well known to the trained artisan that allow the efficient screening of large numbers of samples. See, for example, Cole, JL, Meth Enzymology, 275: 310-328

(1996). Any one of these assays may be suitable in the case of a viral RdRp activity.

One approach for measuring viral RdRp activity in the case of viruses of the Flaviviridae uses a  
5 purified recombinant NS5 protein in an in vitro RdRp assay. For example, Behrens et al., EMBO J., 15: 12-22 (1996) and Lohmann et al., J. Virol., 71: 8416-8428 (1997), describe the baculovirus expression, purification and enzymatic activity of the HCV NS5B  
10 RdRp. The bacterial expression, purification and enzymatic activity of the HCV NS5B RdRp protein has been disclosed in PCT/US96/15571 [WO 97/12033] and by Yuan et al. [Biochem Biophys Res Comm, 232:231-235 (1997)]. In a further example, Collett,  
15 PCT/US99/07404, which is commonly owned with the present application, discloses compositions comprising functional HCV NS5B sequences and their use in identifying compounds useful in the treatment of  
20 hepatitis virus infections. As with the above examples for the HCV RdRp, bacterially-expressed dengue flavivirus NS5 protein has been purified and shown to exhibit RdRp activity [Tan et al., Virology, 216: 317-325 (1996)], as has the NS5B protein of the pestivirus BVDV purified from recombinant baculovirus-infected  
25 cells [Zhong et al., J. Virol., 72: 9365-9369 (1998)].

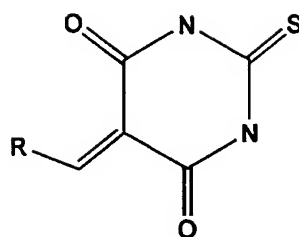
By way of example, the inhibitory activity of candidate antiviral compounds may be determined by using NS5 proteins prepared essentially according to Collett, PCT/US99/07404, in in vitro RdRp assays.  
30 Purified NS5 proteins are incubated in standard RdRp reaction mixtures. Such reaction mixtures generally consist of buffers, salts, cations, reducing agents and the like, as well as nucleoside triphosphates and

an RNA template-primer. Variations in the individual components of such reaction mixtures may be required to accommodate the particular reaction preferences of individual NS5 proteins. Such variations are well known to the trained artisan. Representative compounds falling within formula I, above, as shown in Examples 1-4 and Tables 1 and 2, were evaluated for antiviral activity using this assay. Inhibitory activity by compounds tested was expressed in  $IC_{50}$  values.  $IC_{50}$  values represent the concentration of the compound at which 50% of the RdRp activity is inhibited. The results of the assay for inhibition of RdRp activity in at least one virus of the Flaviviridae family for the compounds tested revealed  $IC_{50}$  values ranging from 0.1  $\mu M$  to about 30  $\mu M$ .

The low concentrations of test compounds required to achieve 50% inhibition of HCV RdRp activity indicate that the method of the invention is effective at inhibiting RNA synthesis by the viral RdRp enzymes involved in Flaviviridae replication.

Although the present invention has been described and exemplified in terms of certain preferred embodiments, other embodiments will be apparent to those skilled in the art. The invention is, therefore, not limited to the particular embodiments described and exemplified, but is capable of modification or variation without departing from the spirit of the invention, the full scope of which is delineated by the appended claims.

TABLE 1

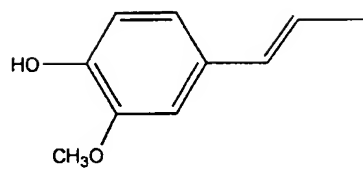


5

R

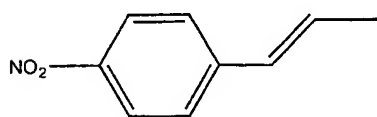
n-propyl

10

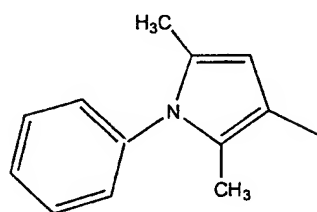


15

20

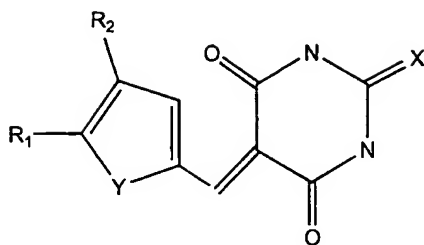


25



30

TABLE 2



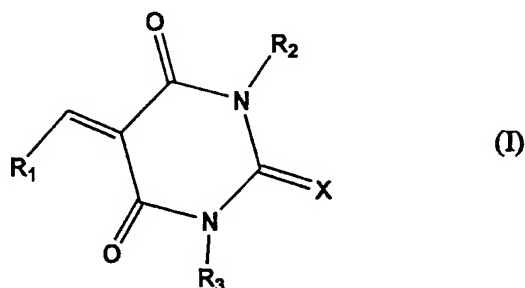
<u>R<sub>1</sub></u>	<u>R<sub>2</sub></u>	<u>X</u>	<u>Y</u>
4-Br-Ph*	H	S	O
4-NO <sub>2</sub> -Ph-	H	S	O
2-Cl-5-NO <sub>2</sub> -PhH	O	O	
4-CO <sub>2</sub> H-Ph	H	S	O
2-CF <sub>3</sub> -Ph-	H	S	O
NaO <sub>3</sub> S-	H	S	O
Br-	H	S	S
2-Cl-Ph	H	S	O
1-CH <sub>3</sub> -5-CF <sub>3</sub> - pyrazol-3-yl	H	S	S
3-Cl-Ph-	H	S	O
NO <sub>2</sub> -	H	S	S
CH <sub>3</sub> -	CH <sub>3</sub> -	S	O
C <sub>2</sub> H <sub>5</sub> -	H	S	O

\* Ph = phenyl (C<sub>6</sub>H<sub>5</sub>-)

## WHAT IS CLAIMED IS:

1. A method of treating infection caused by at least one virus of the Flaviviridae family and disease associated with said infection in a living host having said infection, said method comprising administering to said host a therapeutically effective amount of a compound, or a precursor of said compound, having the formula:

10



wherein X represents a moiety selected from the group consisting of S, O, or N(R<sub>a</sub>), R<sub>a</sub> being hydrogen or alkyl of 1-5 carbon atoms;

- 15 R<sub>1</sub> represents a radical selected from those consisting of an unsubstituted or substituted heterocyclic group, an unsubstituted or substituted bicyclic ring moiety, an unsubstituted or substituted phenyl (C<sub>6</sub>H<sub>5</sub>) group, an unsubstituted or substituted biphenyl (C<sub>6</sub>H<sub>5</sub>-C<sub>6</sub>H<sub>4</sub>) group, an unsubstituted or substituted ω-phenylalkenyl (C<sub>6</sub>H<sub>5</sub>(CH=CH)<sub>n</sub>) group, n being an integer from 1 to 5, an unsubstituted or substituted ω-phenylalkynyl (C<sub>6</sub>H<sub>5</sub>(C≡C)<sub>p</sub>) group, p being an integer from 1 to 5 or an unsubstituted or substituted alkyl group of 1-5 carbon atoms, which may
- 20
- 25

be straight or branched chain, said heterocyclic group being selected from those consisting of furan, thiophene, oxazole, oxadiazole, pyridine, pyrimidine, pyrazole, triazole, pyridazine, 1,3-oxathiolane, thiazole, thiadiazole, imidazole, pyrrole, tetrazole and triazine, said bicyclic ring moiety being selected from those consisting of benzofuran, isobenzofuran, benzothiophene, isobenzothiophene, benzoxazole, benzopyrrole, isoindole, benzpyrazole, quinoline, isoquinoline, 1,2-benzodiazine, 1,3-benzodiazine, 1,2,3-benzotriazole, benzothiazole, benzimidazole, 1,2,3-benzotriazine and 1,2,4-benzotriazine, the heterocyclic group and bicyclic ring moiety substituents being selected from those consisting of alkyl of 1-5 carbon atoms, halogen, alkoxy, hydroxy, nitro, perhaloalkyl, dihaloalkyl, monohaloalkyl, thio, alkylthio, alkylsulfinyl, alkylsulfonyl or an unsubstituted or substituted phenyl group;

$R_2$  and  $R_3$  independently represent hydrogen, an unsubstituted or substituted phenyl group, or  $-(CH_2)_qCOOH$ ,  $q$  being an integer from 1-5, and at least one of said  $R_2$  and  $R_3$  being hydrogen;

the phenyl group substituents, the biphenyl group substituents, the  $\omega$ -phenylalkenyl group substituents and the  $\omega$ -phenylalkynyl group substituents being at least one selected from those consisting of halogen, nitro, carboxy, hydroxy, alkyl of 1-5 carbon atoms, perhaloalkyl, dihaloalkyl, monohaloalkyl, alkoxy, acyloxy, cyano, amino, alkylamino, dialkylamino, sulfonamido, carboxamido, carbalkoxy, thio, alkylthio, alkylsulfinyl and alkylsulfonyl; the alkyl group substituents being at least one selected from those consisting of carboxy, hydroxy, alkoxy, amino,



alkylamino, dialkylamino, thio or alkylthio, and the isomers and pharmaceutically acceptable salts of said compound.

5           2. A method as claimed in claim 1, wherein said compound is administered in unit dosage form containing about 0.001 to about 120 mg of said compound per kilogram of patient body weight per day.

10           3. A method as claimed in claim 2, wherein said unit dosage includes a pharmaceutically acceptable carrier medium.

15           4. A method as claimed in claim 3, wherein a precursor of said compound is administered in the form of a prodrug.

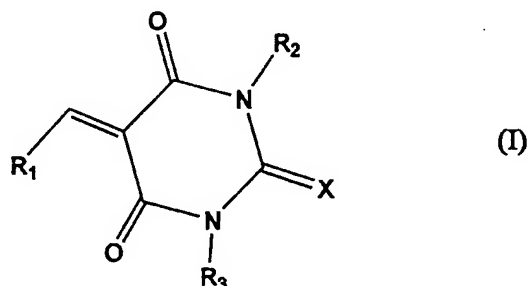
20           5. A method as claimed in claim 1, wherein said compound or a precursor of said compound is administered in combination with at least one supplemental active agent selected from the group consisting of interferons, ribavirin, protease inhibitors, immunoglobulins, immunomodulators, hepatoprotectants, anti-inflammatory agents, antibiotics, antivirals or anti-infectious agents.

30           6. A method as claimed in claim 5, wherein said compound or precursor of said compound and said at least one supplemental active agent are administered simultaneously.

7. A method as claimed in claim 1, wherein said route of administration is selected from the group

consisting of orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, locally or by inhalation.

- 5           8. A method of preventing infection caused by at least one virus of the Flaviviridae family and disease associated with said infection in a living host susceptible to said infection, said method comprising administering to said host a
- 10 prophylactically effective amount of a compound, or a precursor of said compound, having the formula:



- wherein X represents a moiety selected from the group consisting of S, O, or N(R<sub>a</sub>), R<sub>a</sub> being hydrogen or alkyl of 1-5 carbon atoms;
- 15

- R<sub>1</sub> represents a radical selected from those consisting of an unsubstituted or substituted heterocyclic group, an unsubstituted or substituted bicyclic ring moiety, an unsubstituted or substituted phenyl (C<sub>6</sub>H<sub>5</sub>) group, an unsubstituted or substituted biphenyl (C<sub>6</sub>H<sub>5</sub>-C<sub>6</sub>H<sub>4</sub>) group, an unsubstituted or substituted ω-phenylalkenyl (C<sub>6</sub>H<sub>5</sub>(CH=CH)<sub>n</sub>) group, n being an interger from 1-5, an unsubstituted or
- 20
- 25 substituted ω-phenylalkynyl (C<sub>6</sub>H<sub>5</sub>(C≡C)<sub>p</sub>) group, p being

an integer from 1 to 5, or an unsubstituted or substituted alkyl group of 1-5 carbon atoms, which may be straight or branched chain, said heterocyclic group being selected from those consisting of furan, thiophene, oxazole, oxadiazole, pyridine, pyrimidine, pyrazole, triazole, pyridazine, 1,3-oxathiolane, thiazole, thiadiazole, imidazole, pyrrole, tetrazole and triazine, said bicyclic ring moiety being selected from those consisting of benzofuran, isobenzofuran, benzothiophene, isobenzothiophene, benzoxazole, benzopyrrole, isoindole, benzpyrazole, quinoline, isoquinoline, 1,2-benzodiazine, 1,3-benzodiazine, 1,2,3-benzotriazole, benzothiazole, benzimidazole, 1,2,3-benzotriazine and 1,2,4-benzotriazine, the heterocyclic group and bicyclic ring moiety substituents being selected from those consisting of alkyl of 1-5 carbon atoms, halogen, alkoxy, hydroxy, nitro, perhaloalkyl, dihaloalkyl, monohaloalkyl, thio, alkylthio, alkylsulfinyl, alkylsulfonyl or an unsubstituted or substituted phenyl group;

$R_2$  and  $R_3$  independently represent hydrogen, an unsubstituted or substituted phenyl group or  $-(CH_2)_nCOOH$ ,  $n$  being an integer from 1-5, and at least one of said  $R_2$  and  $R_3$  being hydrogen;

the phenyl group substituents, the biphenyl group substituents, the  $\omega$ -phenylalkenyl group substituents and the  $\omega$ -phenylalkynyl group substituents being at least one selected from those consisting of halogen, nitro, carboxy, hydroxy, alkyl of 1-5 carbon atoms, perhaloalkyl, dihaloalkyl, monohaloalkyl, alkoxy, acyloxy, cyano, amino, alkylamino, dialkylamino, sulfonamido, carboxamido, carbalkoxy, thio, alkylthio, alkylsulfinyl and alkylsulfonyl; the alkyl group

substituents being at least one selected from those consisting of carboxy, hydroxy, alkoxy, amino, alkylamino, dialkylamino, thio or alkylthio, and the isomers and pharmaceutically acceptable salts of said compound.

9. A method as claimed in claim 8, wherein said compound is administered in unit dosage form containing about 0.001 to about 120 mg of said compound per kilogram of patient body weight per day.

10. A method as claimed in claim 9, wherein said unit dosage includes a pharmaceutically acceptable carrier medium.

11. A method as claimed in claim 8, wherein a precursor of said compound is administered in the form of a prodrug.

12. A method as claimed in claim 8, wherein said compound or a precursor of said compound is administered in combination with at least one supplemental active agent selected from the group consisting of interferons, ribavirin, protease inhibitors, immunoglobulins, immunomodulators, hepatoprotectants, anti-inflammatory agents, antibiotics, antivirals or anti-infectious agents.

13. A method as claimed in claim 12, wherein said compound or precursor of said compound and said at least one supplemental active agent are administered simultaneously.

14. A method as claimed in claim 8, wherein said  
route of administration is selected from the group  
consisting of orally, rectally, parenterally,  
intracisternally, intravaginally, intraperitoneally,  
5 locally or by inhalation.

10

15

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/20402

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 45/00, 45/05, 47/00

US CL : 424/ 278.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/ 278.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, DOALOG, CAPLUS

search terms: structure search, antiviral, virus, flavivirus, pestivirus, hepatitis

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database CAPLUS, Accession No. 1997:480207, SHIEN et al. 'A study on merocyanine 540 as a photosensitizer for inactivating duck hepatitis B virus', abstract, Chinese Society of Veterinary Science. 1997, Vol. 23, No. 2, abstract.	1-14
Y	US 5,643,891 A (RIDEOUT et al) 01 July 1997, col. 3, lines 5-45.	1-14
Y	US 5,728,684 A (CHENG et al) 17 March 1998, col. 17, lines 5-45.	1-14



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

24 NOVEMBER 1999

Date of mailing of the international search report

09 DEC 1999

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

BRETT L. NELSON

Telephone No. (703) 308-0196